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Amino acid composition of treponemes

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The use of gas-liquid chromatography (GLC) as an analytical tool for identifying and classifying microorganisms has been established (Henis, Gould, and Alexander, 1966; Brooks, Moss, and Dowell, 1969). GLC has been used in this laboratory to study the chemical composition of treponemes in the hope that the information obtained might provide a means of distinguishing the various cultivable strains (Cohen, Moss and Farshtchi, 1970; Farshy, Thomas, and Moss, 1970). The use of GLC in studying the amino acid composition of whole cell hydrolysates of fourteen different cultivable treponemes is described.

Material and methods

The following cultures of treponemes were obtained in lyophilized form from the Venereal Disease Research Laboratory of the Center for Disease Control: Kazan, Kazan 2, Kazan 4, Kazan 5, Kazan 8, Nichols, Reiter, Noguchi, *Treponema calligyra, Treponema ambigua, Treponema microdentium, Treponema refringens, Treponema zuelzerae,* and the N-9 strain of *Borrelia vincentii.* The source, growth medium (which was the same for all cultures), and the techniques employed in cultivating and harvesting these cultures have already been described (Hanson and Cannefax, 1964, 1965). The packed cells from centrifugation were washed six times with physiological saline to remove free amino acids originating from culture medium. The supernatant from the sixth wash was free of amino acids as determined with ninhydrine reagent and GLC. The washed cells were lyophilized.

Aliquots of lyophilized treponeme (20 mg.) were hydrolysed with 2 ml. 6 N HCl in sealed tubes at 105°C. for 16 to 18 hrs. The hydrolysate was extracted twice with ether:hexane (1:1) to remove fatty acids and other organic soluble material. The residual HCl was removed from the extracted hydrolysate by repeated evaporations with methanol on a rotary evaporator. The dried hydrolysate was dissolved in water and placed onto a 1·5 × 15 cm. column of Amberlite* (Mallinckrodt Chemical Works, St. Louis, Mo.) CG-120 ion-exchange resin (100–200 mesh, hydrogen form). Non-amino compounds were removed from the column by washing with approximately 80 ml. distilled water. Amino-compounds were then eluted from the column with 3 N NH_4OH, and the eluent was taken to dryness on a rotary evaporator. The ion-exchange resin was regenerated to the hydrogen-form, and washed, and the next hydrolysate was passed through the column as described above.

Volatile derivatives of amino acids were prepared by the procedures and with the apparatus described by Coulter and Hann (1968), except that trifluoroacetic anhydride (Chemical Research Service, Inc., Addison, Illinois) was substituted for acetic anhydride to produce the N-Trifluoro acetyl (N-TFA)-n-propyl derivative. The derivatives were analysed immediately by GLC or were stored at –20°C. until GLC analysis could be made.

The TFA-n-propyl derivatives were analysed with Barber-Colman Model 5000 Gas Chromatograph (Barber-Colman Company, Rockford, Illinois) equipped with a hydrogen-flame ionization detector and a disc recorder (Series 8000). Samples were analysed on an 8 ft (2·4 m.) U-tube glass column packed with 3 per cent. OV-1 coated on 60–80 mesh Gas-Chrom Q (Applied Science Laboratories, State College, Pennsylvania). Samples were also run on a 6 ft (1·83 m.) glass column packed with 3 per cent. neopentyl glycol succinate (NGS) HI-EFF-3CP coated on 60–80 mesh Gas-Chrom Q (Applied Science Laboratories).

The following operating parameters were used for the OV-1 column: injection temperature, 230°C.; detector temperature, 275°C.; column temperature, 100°C. for 5 min. then 5°C./min. to 250°C.; carrier gas, nitrogen. The NGS column was operated under the same conditions, except that the column temperature was raised from 100 to 250°C. at 6·5°C./min. without an initial isothermal period.

Results

In preliminary studies several procedures and parameters for analysis of amino acids by GLC were evaluated (Gehrke and Stalling, 1967; Pisano and Bronzert, 1969; Coulter and Hann, 1968). In our hands, the method of Coulter and Hann (1968) proved to be the simplest and gave reproducible results. The exact procedures of this method were followed except that the acetylation step was done at 70°C. for 5 min. with 0·4 ml. trifluoroacetic anhydride (25 per cent. in methylene chloride). After the acetylation step, the sample was reduced to 0·5 ml.; 1·0 µlitre of this (N-TFA-n-propyl derivative) was

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analysed by GLC. L-amino acids (Nutritional Biochemicals Corporation, Cleveland, Ohio) were used as standards. Derivatives of individual amino acids and mixtures of amino acids were prepared to establish retention times and relative molar responses to the flame ionization detector (FID). Standards were prepared to contain 2×10^{-9} mole of each amino acid per μlitre of sample injected onto the gas-chromatograph.

The eighteen amino acids listed in Table I were readily converted to volatile derivatives and upon GLC analysis showed sharp, symmetrical peaks. No GLC peaks were observed for arginine and histidine. This is consistent with the findings of Coulter and Hann (1968), who used additional procedures to determine these amino acids by GLC. In the present study, arginine was converted to ornithine by arginase, as described by Coulter and Hann (1968); determinations of histidine were not made.

Fourteen of the eighteen amino acids listed in Table I were resolved by GLC on the OV-1 column. The retention time of ornithine was identical to that of phenylalanine and that of lysine was identical to that of tyrosine on this column; however, these four acids were resolved on the 3 per cent. NGS column (Fig. 1).

The relative molar response of the FID to the eighteen amino acid derivatives are shown in Table I. The response factors were used to calculate the relative molar percentages of amino acids present in the treponemes (Gehrke and Stalling, 1967). The response factors were established in preliminary studies and were checked weekly during the experimental phase.

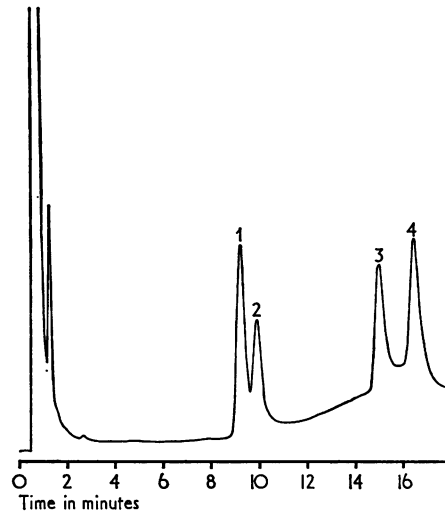

FIG. 1 *Gas-liquid chromatogram run on a 3 per cent. neopentyl glycol succinate column, showing separation of the N-trifluoroacetyl-n-propyl ester derivatives of* (1) *phenylalanine,* (2) *ornithine,* (3) *lysine, and* (4) *tyrosine.*

TABLE I *Amino acid composition of treponemes as determined by gas chromatography*

Organism	*Amino acid*													
	Alanine	*Glycine*	*Serine*	*Threonine*	*Valine*	*Leucine*	*Isoleucine*	*Cysteine*	*Proline*	*Methionine*	*Aspartic acid*	*Phenylalanine*	*Glutamic acid*	*Lysine*
*T. pallidum**														
Kazan	11†	10	4	4	6	12	6	2	4	2	10	10	8	11
Kazan 2	12	6	5	5	6	15	6	T	3	2	15	9	10	6
Kazan 4	10	10	6	7	9	13	5	6	4	4	7	9	6	4
Kazan 5	12	6	5	5	5	12	5	2	4	2	13	8	10	11
Kazan 8	14	11	6	7	8	14	6	T	3	3	11	8	7	2
Nichols	15	11	6	8	9	12	5	T	T	T	12	7	8	7
Reiter	17	9	4	2	7	13	9	T	4	2	12	9	6	6
Noguchi	12	9	5	4	7	11	6	7	5	3	10	8	6	7
T. calligyra	16	8	5	4	10	14	8	T	T	2	12	11	8	2
T. ambigua	15	9	10	10	11	10	3	T	3	8	6	7	6	2
B. vincentii	14	6	6	7	10	13	7	2	6	T	10	6	7	6
T. microdentium	7	6	3	2	8	18	6	2	7	2	12	10	8	9
T. zuelzerae	11	8	5	5	8	14	4	10	2	2	11	10	7	3
T. refringens	13	7	3	6	7	12	4	9	6	2	9	7	6	7

*Non-pathogenic strains
†Relative molar percentage

Shown in Table II are the relative molar percentages of amino acids of fourteen cultivable strains of treponeme. Neither qualitative nor relatively large quantitative differences were observed among the strains. Generally, the most abundant amino acids were alanine, leucine, and aspartic acid; those present in the smallest amounts were generally cysteine, methionine, and proline. Arginine, ornithine, and tyrosine were absent or present in only trace amounts and are not shown in Table II.

TABLE II *Retention times and relative molar response of N-TFA-n-propyl derivatives of amino acids*

Amino acid	*Retention time (min.)**	*Relative molar response (proline = 1·00)*
Alanine	4·25	0·67
Glycine	5·75	0·67
Serine	7·50	0·89
Threonine	7·75	0·78
Valine	8·25	8·83
Leucine	10·50	0·72
Isoleucine	10·75	0·78
Cysteine	12·25	0·50
Proline	13·25	1·00
Hydroxyproline	14·50	1·11
Methionine	17·00	0·95
Aspartic acid	17·25	0·83
Ornithine	19·50	0·83
Phenylalanine	19·50	0·95
Glutamic acid	20·50	1·33
Lysine	22·50	1·00
Tyrosine	22·50	1·06
Tryptophane	31·00	0·72

*Retention time measured to nearest 0·25 min.

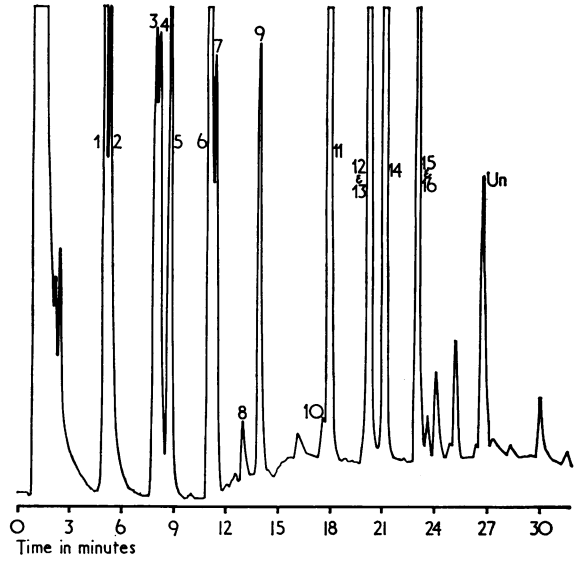

FIG. 2 *Gas-liquid chromatogram (GLC) of amino acids from whole cell hydrolysate of* T. pallidum *strain Kazan 5.*

N-trifluoroacetyl-n-propyl ester derivatives of (1) *alanine,* (2) *glycine,* (3) *serine,* (4) *threonine,* (5) *valine,* (6) *leucine,* (7) *isoleucine,* (8) *cysteine,* (9) *proline,* (10) *methionine,* (11) *aspartic acid,* (12) *phenylalanine,* (13) *ornithine,* (14) *glutamic acid,* (15) *lysine,* (16) *tyrosine.*

GLC analysis was made on 3 *per cent. OV-*1 *column.*

Histidine was not determined, and neither was tryptophane, since it was destroyed during the hydrolysis procedure. Several unidentified peaks occurred in the GLC chromatograms of treponemes (Fig. 2). Most of these were small compared to some of the known amino acids. However, the unidentified peak (retention time of 27 min.) was present in amounts comparable to that of some of the known amino acids and occurred in each of the fourteen strains examined. This peak had a retention time close to, but not indentical with, LL-diaminopimelic acid, a major cell wall amino acid of certain bacteria (Boone and Pine, 1968; Cummins and Harris, 1958; DeWeese, Gerencser, and Slack, 1968). The identity of this compound is currently under study.

Discussion

The above results indicate that amino acid analysis of whole cell hydrolysates provides no useful information for distinguishing among cultivable treponemes. Amino acid analysis with whole cell hydrolysates is, however, a measure of the total amino acids of the cell, and, as such, would reflect only gross differences among organisms. Thus, any difference which might have existed in the amino acid composition of various fractions of the cell (cell wall, cell membrane, lipoprotein, for example) would not have been detected in the present investigation. Further studies to determine the amino acid composition of such fractions are needed; the speed, sensitivity, and simplicity of the GLC technique makes it an ideal analytical tool for such studies.

Summary

The amino acid composition of whole cell hydrolysates of fourteen cultivable treponemes was determined by gas liquid chromatography. Comparison of the relative molar percentages of amino acids revealed no qualitative or large quantitative difference among the strains. Generally, the most abundant amino acids were alanine, leucine, and aspartic acid.

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Composition des tréponèmes en acides aminés

SOMMAIRE

La composition en acides aminés des hydrolysats de cellules entières de 14 tréponèmes cultivables a été déterminée par chromatographie gaz-liquide. La comparaison des pourcentages moléculaires relatifs des acides aminés n'ont pas révélé de différence qualitative ou de différence quantitative importante entre les souches. Généralement, les acides aminés les plus abondants étaient l'alanine, la leucine et l'acide aspartique.